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Light Harvesting in a Fluctuating Antenna

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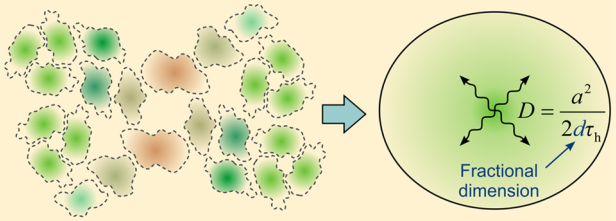
Supporting Information

ABSTRACT: One of the major players in oxygenic photosynthesis, photosystem II (PSII), exhibits complex multiexponential fluorescence decay kinetics that for decades has been ascribed to reversible charge separation taking place in the reaction center (RC). However, in this description the protein dynamics is not taken into consideration. The intrinsic dynamic disorder of the light-harvesting proteins along with their fluctuating dislocations within the antenna inevitably result in varying connectivity between pigment–protein complexes and therefore can also lead to nonexponential excitation decay kinetics. On the basis of this presumption, we propose a simple conceptual model describing excitation diffusion in a continuous medium and accounting for possible variations of the excitation transfer rates. Recently observed fluorescence kinetics of PSII of different sizes are perfectly reproduced with only two adjustable parameters instead of the many decay times and amplitudes required in standard analysis procedures; no charge recombination in the RC is required. The model is also able to provide valuable information about the structural and functional organization of the photosynthetic antenna and in a straightforward way solves various contradictions currently existing in the literature.



1. INTRODUCTION

An absolute majority of living beings inhabiting the Earth vitally depend on the outstanding ability of some organisms to absorb the electromagnetic radiation coming from the Sun and to store it in the form of chemical energy, a phenomenon known as photosynthesis. Over billions of years of evolution, various photosynthetic organisms have developed different photosynthetic apparatus. Being completely diverse in their structure, all these apparatus are designed in a very similar way: the major part of the photosynthetic membrane—the so-called light-harvesting antenna—is composed of pigment molecules [e.g., (bacterio)chlorophylls and carotenoids] usually bound to a protein scaffold.[1,2] The mutual arrangement of these pigment–protein complexes, as well as their spectroscopic properties, ensures an optimal absorption of the incoming photons and can lead to extremely efficient (up to 99%) delivery of the generated electronic excitations to a reaction center (RC), where excitation energy is stabilized in the form of a transmembrane electrochemical potential necessary for the subsequent stages of photosynthesis.[1,2] Specific molecular mechanisms responsible for such high efficiency of excitation energy transfer through the light-harvesting antenna are still not fully understood. The phenomenon becomes even more remarkable if one takes into account the intrinsic structural disorder of biological systems and continuous spatial rearrangement of the pigment–protein complexes within the photosynthetic membrane taking place during state transitions, nonphotochemical quenching, and protein repair (see, e.g., refs 3, 4 for recent reviews).

Among all supramolecular photosynthetic complexes, photosystem II (PSII) from green plants, algae, and cyanobacteria has always attracted a lot of attention due to its outstanding physiological significance: the reaction center of PSII uses water as primary electron donor and, as a byproduct of photochemical reactions, generates molecular oxygen indispensable for all aerobic organisms.[2] Occupying a large part of the thylakoid membrane, PSII supercomplexes are present mainly in dimeric form.[5,6] The schematic mutual arrangement of pigment–protein complexes in PSII is presented in Figure 1b. The core of PSII—the minimal functionally independent structural element that is able to perform water splitting—consists of an RC that is bound to the core antenna proteins (CP43 and CP47). The PSII core is surrounded by the major trimeric light-harvesting antenna complexes, LHCII, which are coupled to the core via the minor monomeric complexes CP24, CP26, and CP29. The LHCII complexes are not only responsible for efficient light harvesting under low illumination conditions but also take part in the photoprotection of the thylakoid membrane against intense light via the process of nonphotochemical quenching.[5] Moreover, they exhibit a high mobility level and can, when needed, migrate within the

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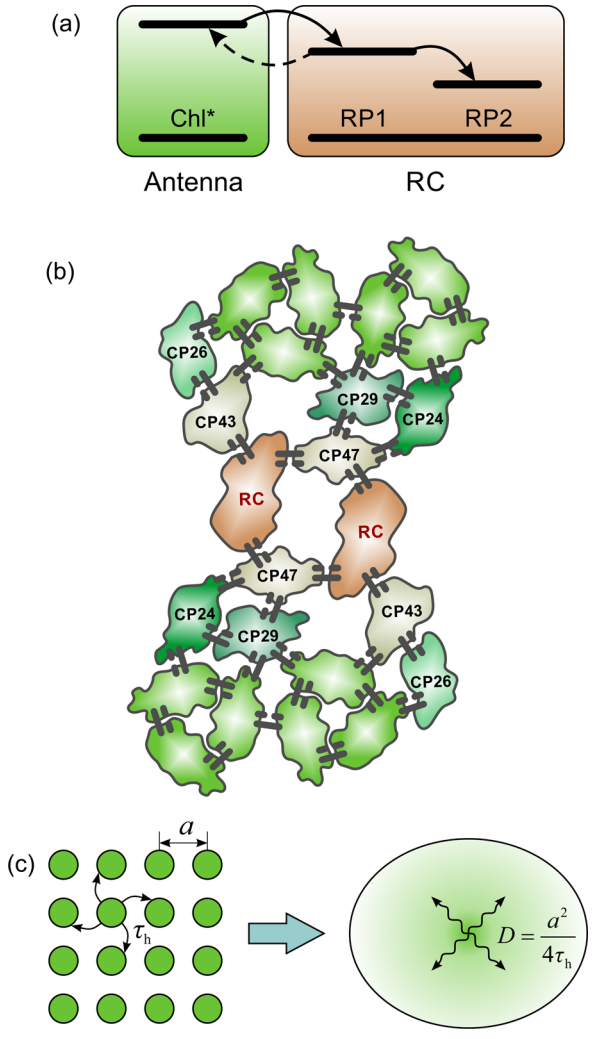

Figure 1. (a) Trap-limited ERPE model of excitation dynamics in PSII assumes an instantaneous Boltzmann equilibration between the PSII excited state and first radical-pair state in RC. (b) Schematic structure of the largest purified PSII supercomplexes ($C_2S_2M_2$, notated below as B11) exhibiting its dimeric nature. LHCII trimers are presented in light green and are not labeled. Thick solid gray bars indicate pathways of intercomplex excitation energy transfer, as used in the coarse-grained model; on the other hand, broken gray lines demonstrate that due to structural fluctuations of PSII (occurring on a time scale longer than excitation mean lifetime) the efficiency of these pathways is not constant, which results in the varying rates of the excitation energy transfer. This assumption is the prerequisite of the current work. (c) Switching from the CG model to the diffusional limit, when excitation migration through the antenna is described by a single diffusion equation [two-dimensional (d = 2) case].

thylakoid membrane, separating from PSII and binding to photosystem I.[8]

Crystal structures of LHCII,[9] CP29,[10] and PSII core complexes,[11] obtained with a resolution higher than 3 Å, provided essential information about the structural organization of these complexes. A series of time-resolved spectroscopic measurements, accompanied by structure-based theoretical modeling,[12−17] contributed to a deeper insight into details of excitation energy transfer dynamics in PSII complexes. Recent advances in understanding of these topics as well as the internal organization of PSII from the level of individual complexes to the entire thylakoid membrane have been reviewed (see refs 4, 18, 19). However, a complete detailed model of light-harvesting in PSII is still a challenge because it requires one not only to know the *static* spectroscopic properties and precise mutual arrangement of the pigment molecules but also to understand how the intrinsic disorder and *dynamic* fluctuations of the considered biological system can influence these properties and the overall excitation dynamics.[4]

Prior to making a first attempt toward formulating such a model of a fluctuating antenna, we first shortly discuss currently existing theoretical models of excitation energy transfer within the photosynthetic antenna and outline several recent experimental studies that have revealed some contradictions in our current understanding of light-harvesting processes in biological systems. As a possible solution for this inconsistency, we propose an alternative way to describe complex multi-exponential fluorescence decay kinetics by using just a few parameters.

2. NEW EXPERIMENTS REVEAL AN INCONSISTENCY IN THE EXISTING MODELS

2.1. Trap-Limited vs Migration-Limited Regime. Starting from the very first picosecond time-resolved fluorescence measurements, the excitation radical-pair equilibrium (ERPE) model has been suggested and widely used for interpretation of the fluorescence decay kinetics of PSII.[20−23] In this simple model, summarized in Figure 1a, it is assumed that the initial excitation instantaneously equilibrates over the whole light-harvesting system, so that the overall process of excitation decay depends only on the rate of charge separation in the RC and the energy difference between the thermally equilibrated excited state of PSII and first radical-pair (RP) state in the RC. Therefore, according to this approach, the excitation dynamics is a *trap-limited* process. However, this model contradicts the results of singlet−singlet exciton annihilation studies on LHCII aggregates:[24−26] in the case of instantaneous excitation equilibration over the whole quenched aggregate, the normalized transient absorption kinetics would be almost independent of the intensity of initial excitation,[27] as opposed to the actual observations. In fact, fluorescence measurements,[28] as well as structure-based calculations[15,29] of PSII core, predicted a slow (at least several tens of picoseconds) energy transfer between the core antenna complexes (CP43/CP47) and RC, suggesting a *transfer-to-trap limiting* (TTL) regime.

Later, the models were extended by taking into account the structural arrangement of the pigment−protein complexes of PSII in a superlattice form, as determined by means of electron microscopy.[6,30] The resulting coarse-grained (CG) model[31−34] (see Figure 1b) assumed an instantaneous excitation equilibration within a given antenna complex only, whereas the intercomplex excitation migration toward the RC was explicitly taken into account and described by a single parameter, the mean excitation hopping time (τ_h). Depending on the antenna size and the excitation migration rate, this model can either present some perturbation to the trap-limited model[35] or describe the excitation dynamics as a *migration-limited* process. In fact, the mean excitation lifetime, $\langle\tau\rangle$, can be split into three terms describing excitation migration through the antenna (τ_{mig}) and its subsequent delivery from the core antenna complexes to the RC (τ_{del}) followed by the photochemical excitation trapping (τ_{trap}):[1]

$$\langle\tau\rangle = \tau_{mig} + \tau_{del} + \tau_{trap} \quad (1)$$

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All these terms depend on the antenna size and reflect the effective timescales of the corresponding processes. Therefore, their relative magnitudes define which regime is the most appropriate.

It was found[32] that excitation of the outer antenna in PSII membranes (so-called BBY particles, on average containing 2.5 LHCII trimers per RC) leads to longer excited-state lifetimes than direct excitation of the core. The excitation kinetics in the entire thylakoid membranes (with four LHCII trimers per RC)[36] suggested that at least 50% of the overall lifetime of excited-state in PSII arises from the migration term. Recently, a first attempt was made to describe the excited-state dynamics within the PSII supercomplex with a combined generalized Förster/modified Redfield approach[37] while merging existing high-resolution crystal structures of individual pigment–protein complexes with a low-resolution image of the PSII supercomplex. This consideration demonstrated the dominating role of the first two terms in eq 1 that are comparable one to another. However, this conclusion should still be considered rather cautiously since it was obtained for a single static arrangement ("*snapshot*") of the pigment–proteins and did not take into account any *dynamic* reorganization of PSII structure.

2.2. Novel Experimental Data. Recently performed time-resolved fluorescence measurements of the excitation decay kinetics of variably sized PSII supercomplexes[34] provided additional data to test the existing models for their response to the changes in antenna size. However, these measurements also cast some doubts about the contemporary fundamental understanding of light-harvesting processes in PSII. First of all, it was shown that the mean excitation lifetime in PSII increases quasi-linearly with the number of chlorophyll (Chl) pigments present in the antenna (see Figure 2). However, upon extrapolation to the systems with vanishing antenna size, the mean excitation lifetime did not converge to zero but to some finite value between 50 and 80 ps. Contrarily, ERPE, CG, and TTL models predict a nearly linear, zero-crossing relationship between both the migration (τ_{mig}) and delivery (τ_{del}) terms of the mean lifetime (see eq 1) and the number of antenna pigments.[1] Therefore, according to the mentioned models, the extrapolated values might be only related to the intrinsic charge separation time scale and for the open RCs obviously are too slow.

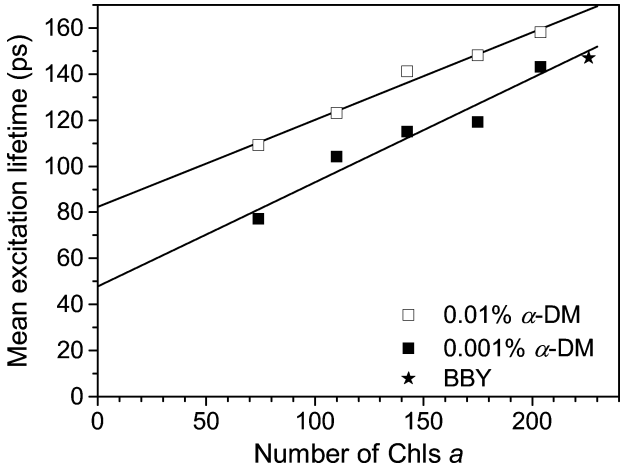

Figure 2. Excited-state mean lifetimes vs number of Chl *a* molecules per PSII of different antenna size being solubilized in 0.01% and 0.001% α-DM as well as in BBY particles. The lines represent linear fits that do not approach zero as the number of Chl *a* pigments vanishes. Adapted from ref 34.

Another important issue regarding the excitation energy transfer in PSII is the multiexponential fluorescence decay kinetics observed in nearly all sample preparations independently of the antenna size.[31,34,38] All the models discussed so far predict almost monoexponential kinetics in the case of irreversible charge separation in the RCs. Therefore, in order to deal with such multiexponential behavior, all the models had to assume energy equilibration between the radical pair state in the RC and the neighboring antenna pigments. The RC is then treated as a trap with nonzero possibility of charge recombination. In order to properly describe fluorescence decay kinetics for all the experimental data, it had to be assumed that the intrinsic rate of charge separation in the RC is strongly dependent on the size of the distant peripheral antenna.[34] Furthermore, the commonly used postulation of radical pair equilibration contradicts another recent claim that initial charge separation in the RC is indeed virtually irreversible,[39] which was also proposed in other studies.[15,37] If so, a fundamental question about the basic properties of the excitation energy dynamics in PSII arises: if not the radical pair equilibration in the RC, then *what* is the origin of the nonmonoexponential fluorescence decay kinetics? The possible answer is concealed in the fluctuating properties of light-harvesting antenna, as has been determined by means of electron microscopy.[4,6]

It is well-known that in disordered systems nonexponential behavior sometimes may arise due to statistical averaging of the exponential decay kinetics over some particular distribution of the rate constants[40] and site energies[15] or when dynamic disorder is taken into account.[41] A relatively high level of intrinsic disorder along with the mobility of the light-harvesting antenna[4,7] suggest that the internal structure of PSII is not static, so that the distances between the pigment–protein complexes and their mutual orientation constantly vary over time, causing a temporal fluctuation of the interpigment coupling strength and, as a result, of the interpigment excitation energy transfer rate. It was found recently that even a single pigment–protein unit with a seemingly stable internal structure exhibits some intrinsic conformational dynamics, forcing it to switch constantly and randomly between different states that are completely opposite in nature and represent light-harvesting and quenched states.[42−44] At the level of the entire PSII, such intrinsic fluctuations along with the macroscopic reorganization of the antenna complexes might lead to even more drastic and unexpected results. This implies that any *static* model of the PSII antenna described by a fixed set of pigment pools and constant excitation hopping times is most likely not sufficient. For a proper description of light-harvesting processes, the dynamic fluctuations and reorganization of the whole antenna should be taken into consideration. However, this is a challenging task requiring precise time-resolved experimental structural data as well as computationally expensive molecular dynamics simulations. Therefore, we propose a simple *conceptual* model of excitation diffusion in a continuous medium taking into account possible variations of intercomplex connectivity. We show that even such an oversimplified approach can perfectly describe the multiexponential fluorescence decay kinetics without assuming the radical pair equilibration in the RC. Moreover, this model also naturally solves the problem that the excitation mean lifetime does not extrapolate to zero in the case of vanishing antenna size.

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Additionally, it provides some information about the structural organization of the photosynthetic antenna (either planar or stacked) as well as the disturbances in the intercomplex connectivity reflected by the obtained fractional dimensionality of PSII.

3. FLUCTUATING ANTENNA MODEL

As discovered in recent studies, a contribution from long-living quantum coherence might influence interpigment excitation dynamics on a fast, subpicosecond time scale.[45−47] On the other hand, these quantum effects are usually much less pronounced or even disappear on the longer time scale of excitation migration through the whole PSII, which takes from tens to hundreds of picoseconds. Therefore, excitation energy transfer in the light-harvesting antenna is often described by the Pauli master equations (see, e.g., ref 48), which is also the case for the coarse-grained model[31−34] and the detailed domain model:[37]

$$\frac{\mathrm{d}}{\mathrm{d}t}p_i(t) = \sum_j k_{j\to i}p_j(t) - \sum_j k_{i\to j}p_i(t) \tag{2}$$

Here $p_i(t)$ is the time-dependent probability for the excitation to reside on the ith pigment−protein complex (in the CG model[31]) or in the ith domain of strongly coupled pigments (in the domain model[37]) and $k_{i\to j}$ is the effective rate of excitation transfer from the ith to the jth complex (or domain), intrinsically taking into account all the quantum effects being relevant within the given complex or domain. In the ideal *square lattice* (Figure 1c), where excitation transfer between adjacent domains only (separated by distance a) is taken into account (with the constant excitation hopping time $\tau_h = k_{i\to j}^{-1}$), the system of the master equations (eq 2) can be well-approximated by a single equation describing excitation diffusion in a continuous two-dimensional medium:

$$\frac{\partial}{\partial t}p(x, y, t) = D\nabla^2 p(x, y, t) \tag{3}$$

Here $\nabla^2 = (\partial^2/\partial x^2) + (\partial^2/\partial y^2)$ is the two-dimensional Laplace operator, $p(x,y,t)$ is the excitation density at the point (x,y) at time t, and $D = a^2/(4\tau_h)$ is the excitation diffusion constant for this particular square lattice. In this work, we use a similar approach to describe excitation energy transfer through PSII by a single diffusion equation. However, to account for the varying intercomplex connectivity, additional assumptions by formulating a *fluctuating light-harvesting antenna model* are needed.

3.1. Model Formulation. As already discussed, in the case of irreversible charge separation, the observed multiexponential excitation decay kinetics in PSII become hardly comprehensible. It was shown recently that random spatial distribution of static traps can in principle explain similar multiexponential fluorescence decay kinetics observed in aggregates of LHCII complexes.[27] In the case of PSII, the location of the excitation trap—the RC—is fully determined. However, due to the flexibility of the whole system, the interconnectivity between different subunits remains unresolved, and the fluctuating motion of the protein scaffold can result in varying arrangement of the network of excitation transfer pathways. As will be shown below, the averaging over the ensemble of these distinct pathways also leads to nonexponential excitation decay kinetics.

To deal with a random distribution of excitation transfer pathways, with a zero-order approximation one can simplify the task and neglect the discrete nature of mutual arrangement of Chl molecules by considering excitation diffusion in a continuous medium. Such a simplification and the lack of connectivity in some antenna points can then be (at least partially) accounted for by allowing the dimensionality of that continuous medium to be described by a *fractional* number, d.[1,27,49] This approach extends previous studies on nonexponential excitation decay kinetics in one-dimensional systems arising from the presence of randomly distributed traps.[50,51]

We assume that the initial pointlike excitation is completely trapped after diffusing for some random distance R, which mimics some particular length of the excitation pathway toward the RC. The time evolution of the excitation in such a system can be described by a diffusion equation similar to eq 3

$$\frac{\partial}{\partial t}p(\vec{r}, t|R) = D\nabla_d^2 p(\vec{r}, t|R) \tag{4}$$

with the initial condition $p(\vec{r},t=0|R) = \delta(\vec{r})$ and boundary condition given by

$$p(\vec{r}, t|R)|_{|\vec{r}|=R} = 0$$

Here $p(\vec{r},t|R)$ is the density of the survived excitation at the time moment t, parametrically depending on R; D is the diffusion constant; and $\delta(\vec{r})$ is the Dirac delta function determining the initial pointlike excitation, corresponding to the excitation of some particular complex in the CG model. Due to the spherical symmetry of the excitation migration, the Laplacian ∇_d^2 depends on the distance r only, and in a d-dimensional system it is defined as follows:[52]

$$\nabla_d^2 = \frac{\partial^2}{\partial r^2} + \frac{d-1}{r}\frac{\partial}{\partial r} \tag{5}$$

By separating the variables r and t, the solution of eq 4 can be given by

$$p(\vec{r}, t|R) \equiv p(r, t|R) = \sum_n f_n(r|R)\exp[-\varepsilon_n(R)t] \tag{6}$$

where $f_n(r|R)$ and $\varepsilon_n(R)$ are the eigenfunctions, obeying the boundary condition

$$f_n(r|R)|_{r=R} = 0 \tag{7}$$

and the eigenvalues of the equation

$$D\nabla_d^2 f_n + \varepsilon_n f_n = 0 \tag{8}$$

respectively. By substituting the Laplacian from eq 5 into eq 8 we obtain the well-known Bessel equation

$$r^2 f_n''(r|R) + r(d-1)f_n'(r|R) + r^2\frac{\varepsilon_n}{D}f_n(r|R) = 0 \tag{9}$$

from which the total solution of eq 4 can be written in an analytical form as

$$p(r, t|R) = \sum_{n=1}^{\infty} C_n r^{1-d/2} J_{d/2-1}\left(\xi_{d/2-1}^{(n)}\frac{r}{R}\right) \times \exp[-(\xi_{d/2-1}^{(n)}/R)^2 Dt] \tag{10}$$

(see part I of the Supporting Information for details). Here $J_{d/2-1}(\xi)$ is the Bessel function of the first kind and fractional (either positive or negative) order $d/2-1$, while $\xi_{d/(2-1)}^{(n)}$ stands for the nth zero of that Bessel function, obeying $J_{d/2-1}(\xi_{d/2-1}^{(n)}) = 0$. The amplitudes C_n in eq 10 are given by eq S4 in the Supporting Information. Finally, by integrating eq 10 over the whole volume $V(R)$ confined by the spherical boundary of the

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radius R, we obtain the total population of the excitation survived in the system until the time t:

$$P(t|R) = \int_{V(R)} p(r, t|R)\, \mathrm{d}V = \frac{4}{2^{d/2}\Gamma\left(\frac{d}{2}\right)} \sum_{n=1}^{\infty} \frac{(\xi_{d/2-1}^{(n)})^{d/2-2}}{J_{d/2}(\xi_{d/2-1}^{(n)})} \times \exp[-(\xi_{d/2-1}^{(n)}/R)^2 Dt] \quad (11)$$

Here $\Gamma(d/2)$ stands for the gamma function. As expected, the proper choice of the coefficients C_n in eq 10 ensures that $P(t=0|R) = 1$.

3.2. Averaging Kinetics. The excitation decay kinetics presented by eq 11 was obtained by assuming that the excitation has been diffusing in an unperturbed way inside the d-dimensional sphere of radius R and volume V. In other words, this equation corresponds to some specific excitation pathway of the length R on its route toward the RC. Averaging over all possible pathways in this case can be performed similarly, as it was done while analyzing excitation dynamics in the LHCII aggregates with randomly distributed traps.[27] Free diffusion inside the sphere of radius R means that there were no additional traps (i.e., RC) inside that sphere. We assume that the traps (or, in other words, the path lengths to the RC) are distributed according to Poisson statistics, so that the probability to obtain k traps inside some volume V is given by

$$W^{(k)}(V) = \frac{\bar{N}^k}{k!} \mathrm{e}^{-\bar{N}}$$

where $\bar{N} = cV$ is the average number of the traps in the volume V, and c is an average concentration of the traps. Then the probability to find no traps inside the sphere of volume V can be written as $W^{(0)}(V) = \mathrm{e}^{-cV}$. This quantity can be treated as the (non-normalized) probability density $\omega(V)$ for obtaining the system with some particular value of V. After normalizing the total probability $\int_0^\infty \omega(V)\, \mathrm{d}V$ to unity we obtain

$$\omega(V) = c\mathrm{e}^{-cV} \quad (12)$$

This probability density can be used to average the kinetics given by eq 11 over different realizations of R (or V):

$$\bar{P}(t) = \int_0^\infty P(t|R(V))c\mathrm{e}^{-cV}\, \mathrm{d}V \quad (13)$$

where the relationship between R and V is given in the Supporting Information (see eq S3).

Although presenting an analytical expression of the survival probability, eq 11 is still rather complex for ordinary usage while analyzing experimental excitation kinetics. Nevertheless, its nonexponential behavior is evident; moreover, this behavior remains even in the asymptotics $t \to \infty$, when the contribution from the single term with the smallest eigenvalue $\varepsilon_1 = D(\xi_{d/2-1}^{(1)}/R)^2$ dominates:

$$\bar{P}(t) \simeq A_d(c^{2/d}Dt)^{d/(2d+4)} \exp[-\kappa_d(c^{2/d}Dt)^{d/(d+2)}] \quad (14)$$

(see part II of the Supporting Information for details and expressions for the coefficients κ_d and A_d). More importantly, this asymptotic form of excitation decay kinetics reveals that in fact our model contains just two undetermined parameters: the dimension of the system, d, and a simple function of the diffusion coefficient and the mean concentration of the traps, $Dc^{2/d}$, which both can be fitted to the experimentally obtained excitation relaxation kinetics in PSII.

3.3. Excitation Mean Lifetime. Differently from the kinetics, for which we can obtain a rather simple approximate analytical expression describing the excitation decay only at longer times, the average lifetime of the excitation can be calculated directly from eq 11:

$$\tau(R) = \int_0^\infty P(t|R)\, \mathrm{d}t = \frac{R^2}{D} \frac{4}{2^{d/2}\Gamma\left(\frac{d}{2}\right)} \sum_{n=1}^{\infty} \frac{(\xi_{d/2-1}^{(n)})^{d/2-4}}{J_{d/2}(\xi_{d/2-1}^{(n)})}$$

Interestingly, the total sum of this very complex infinite series converges to a very simple expression

$$\tau(R) = \frac{R^2}{2dD} \quad (15)$$

i.e., the mean lifetime of the excitation diffusing inside the sphere is just equal to the *diffusion time* needed for the excitation to diffusively reach the distance R. The averaging over various radii R is performed in the same way as it was done for the kinetics:

$$\langle\tau\rangle = \int_0^\infty \tau(R(V))\, \omega(V)\, \mathrm{d}V = \frac{\Gamma\left(\frac{2}{d}\right)}{\pi d^2 D}\left[\frac{\Gamma\left(1+\frac{d}{2}\right)}{c}\right]^{2/d} \quad (16)$$

This equation gives a rather simple expression for the excitation mean lifetime and, again, depends on the same two parameters, d and $Dc^{2/d}$.

3.4. Relationship to the Coarse-Grained Model. Turning back to the structural arrangement of the pigment–protein complexes in PSII, the concentration c of the RCs can be expressed as

$$c \simeq \frac{1}{Na^d} \quad (17)$$

where N is the average number of complexes per RC, and a^d is the average volume of those complexes (in d-dimensional space; cf. Figure 1c for the 2-dimensional case). Similarly, the diffusion constant D is related to the macroscopic excitation hopping time, τ_h, between adjacent complexes in a similar way as was defined in eq 3:

$$D \simeq \frac{a^2}{2d\tau_h} \quad (18)$$

By substituting eqs 17 and 18 into eq 16 we obtain

$$\langle\tau\rangle = \tau_h N^{2/d} \frac{2\Gamma\left(\frac{2}{d}\right)}{\pi d}\left[\Gamma\left(1+\frac{d}{2}\right)\right]^{2/d} \quad (19)$$

which for integer dimensions yields

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$$\langle \tau \rangle = \begin{cases} \frac{1}{2}\tau_h N^2 & d = 1 \\ \frac{1}{\pi}\tau_h N \approx 0.32\tau_h N & d = 2 \\ \Gamma\left(\frac{2}{3}\right)\left(\frac{N}{\sqrt{6}\pi}\right)^{2/3}\tau_h \approx 0.35\tau_h N^{2/3} & d = 3 \end{cases}$$

We see a strictly linear dependence of $\langle \tau \rangle$ on N only in the case of the ideally two-dimensional arrangement of the pigment–protein complexes. Moreover, for any dimensionality d the mean lifetime approaches zero as $N \to 0$.

4. MODELING RESULTS

4.1. Fitting Fluorescence Decay Kinetics. Recent picosecond fluorescence measurements of PSII supercomplexes with largely varying antenna sizes have revealed the importance of the usually redundant interconnectivity between various pigment–protein complexes, ensuring the robustness of the system, and the strong dependence of the excitation decay kinetics on the antenna size.[34] There the fluorescence decay kinetics, originating from five different fractions of PSII supercomplexes being solubilized using two different detergent concentrations, were presented. These PSII fractions, having been labeled as core, B8, B9, B10, and B11, on average contained N = 3, 5.5, 7.8, 10, and 12 pigment–protein complexes per RC, respectively (see Figures 1b and 3 for schematic structures). Following the original suggestion to ignore the longest nanosecond-scale time components in the fluorescence decay traces (arising probably from closed RCs, free Chls or separated antenna complexes),[34] we used the experimentally measured kinetics to test whether our model, containing just two free parameters, can fully reproduce the multiexponential behavior without assuming the presence of additional radical-pair states in the RC. For comparison, we have also analyzed fluorescence kinetics obtained from BBY particles,[31,32] on average containing 13.2 complexes per RC.

For calculations we used the first 30 terms in the infinite series of eq 11, all being averaged according to eq 13. The resulting best-fitted fluorescence decay kinetics are presented in Figure 4, and the corresponding fitting parameters are listed in Table 1. In the same table we also present the mean excitation lifetimes calculated according to eq 16 using the obtained model parameters and compare them with the actual experimental values.[29,31] As shown in Figure S1 in the Supporting Information, the excitation decay kinetics approaches its asymptotic behavior (eq 14) for times $t \gtrsim 200$ ps.

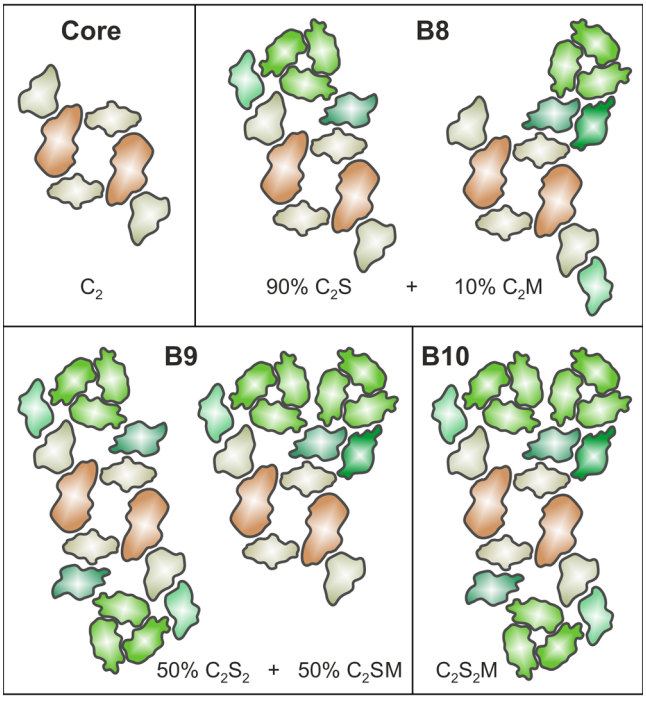

Figure 3. Schematic structures of the core and B8–B10 PSII supercomplexes. Colors and relative positions of the pigment–protein complexes are the same as in Figure 1b, where the structure of B11 particles is shown.

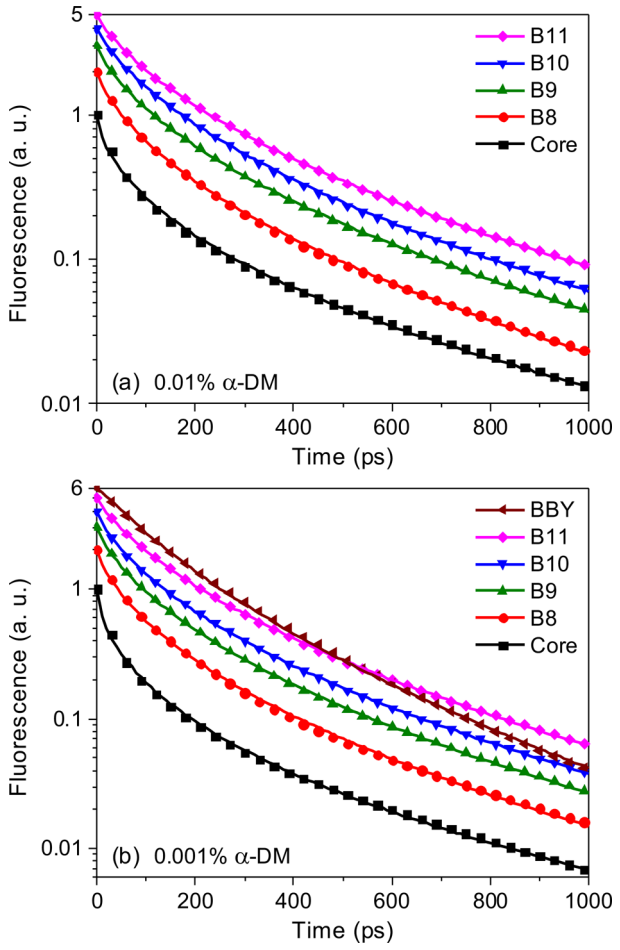

Figure 4. Experimental (symbols) and simulated (lines) fluorescence decay kinetics in PSII of different sizes solubilized in 0.01% (a) and 0.001% (b) α-DM. For comparison, panel b also shows kinetics in BBY particles. All the simulated curves were calculated using the fitted parameters presented in Table 1. For visual clarity, fluorescence kinetics in B8, B9, B10, B11, and BBY supercomplexes were multiplied by integer numbers 2, 3, 4, 5, and 6, respectively.

4.2. Mean Intercomplex Hopping Time. As follows from eqs 17 and 18, the fitted values of the product $Dc^{2/d}$ depend both on the mean excitation intercomplex hopping time, τ_h, and the number of antenna complexes per RC, N, since $[Dc^{2/d}]^{-1} = 2d\tau_h N^{2/d}$. In fact, the numbers N represent the amount of subunits participating in the excitation energy transfer and therefore are to some extent an arbitrary choice: they can be

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Table 1. Fitted Model Parameters and Mean Excitation Lifetimes Obtained for PSII of Various Size for Different Concentrations of Detergent[a]

	model parameters		⟨τ⟩ (ps)	
PSII	d	$[Dc^{2/d}]^{-1}$ (ns)	model	experiment
BBY	2.300(7)	2.103(9)	147	147
		0.01% α-DM		
core	1.277(9)	0.759(12)	112	109
B8	1.569(4)	1.170(6)	124	123
B9	1.618(3)	1.395(4)	142	141
B10	1.686(3)	1.544(5)	149	148
B11	1.687(2)	1.656(4)	159	158
		0.001% α-DM		
core	1.180(9)	0.446(8)	76	77
B8	1.543(5)	0.959(7)	104	104
B9	1.603(3)	1.125(4)	116	115
B10	1.615(3)	1.170(4)	119	119
B11	1.781(3)	1.595(4)	144	143

[a]Mean excitation lifetimes are calculated from the fitted model parameters using eq 16 and are compared with the experimental ones.[32,34] Numbers in parentheses represent the uncertainty of the obtained model parameters corresponding to 95% confidence interval.

chosen to correspond to the number of pigment–protein complexes, to the number of the domains made by strongly coupled pigments, or even to the total number of Chl molecules. Then the corresponding numerical value of τ_h will reflect the mean intercomplex, interdomain, or interpigment excitation hopping time, averaged over the whole PSII.

The interdependence between N and τ_h for various fractions of PSII is presented in Figure 5. The filled stars in Figure 5 correspond to the actual number of the pigment–protein complexes per RC, with their ordinates then representing the mean intercomplex excitation transfer times.

5. DISCUSSION

Turning back to Figure 4, we would like to stress an outstanding result revealed by our simulations: despite being exceptionally simple and dealing merely with an ordinary excitation diffusion in a continuous medium, our model, intrinsically taking into account the fluctuating nature of the intra- and intercomplex connectivity, is able to exactly reproduce complicated multiexponential fluorescence decay kinetics in PSII supercomplexes. In contrast to all the existing modifications of the ERPE model, this complex behavior of the fluorescence decay is explained by using just two parameters, both having a simple physical meaning and being in agreement with the current knowledge of excitation dynamics in photosynthetic antennae of PSII.

First of all, the obtained dimensionality of the PSII core is very close to 1, as it is expected for the chain arrangement of CP43 and CP47 core antenna complexes around the RCs. On the other hand, the fractional dimension $1.5 < d < 2$ observed in B8–B12 types of PSII corresponds to the perturbed coordination within the planar distribution of light-harvesting complexes. In other words, it simply reflects the presence of void regions and the lack of connectivity between some complexes arranged into two-dimensional aggregates. Contrarily, the obtained larger dimension for the BBY particles, $d \approx 2.3$, reflects the known quasi-3D, or stacked, structure of photosynthetic thylakoid membranes.[5] A similar quasistacked

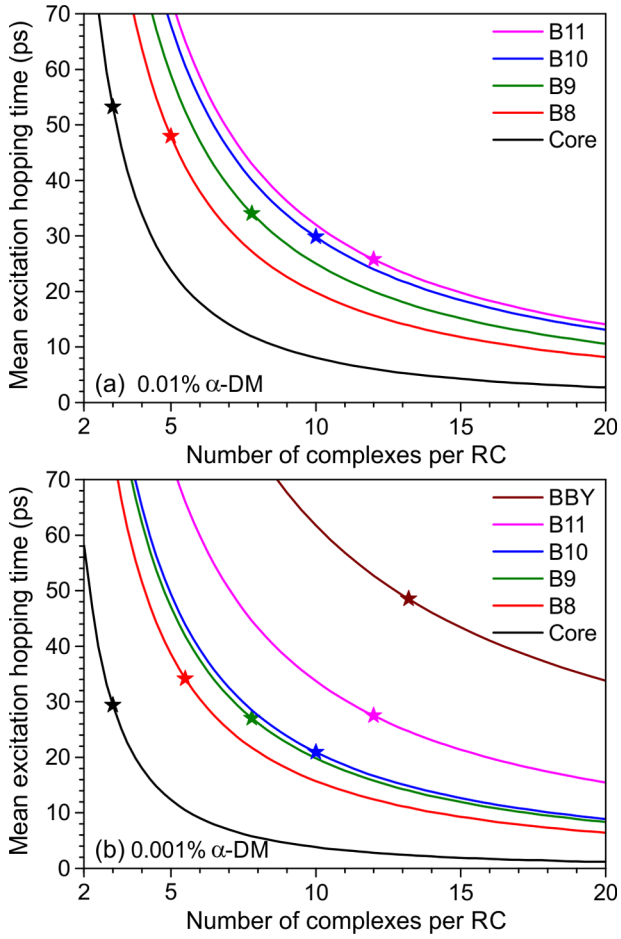

Figure 5. Mean intercomplex hopping time vs number of pigment complexes per RC, obtained for different fractions of PSII solubilized in 0.01% (a) or 0.001% (b) α-DM and ensuring the same fitting parameters as listed in Table 1. For comparison, panel b also shows data for BBY particles. Stars indicate the hopping times corresponding to the actual number of pigment–protein complexes per RC in the corresponding PSII supercomplex (N = 3, 5.5, 7.8, 10, 12, and 13.2 for the core, B8, B9, B10, B11, and BBY, respectively).

distribution of antenna complexes was also assumed in our previous coarse-grained modeling of the BBY complexes.[33]

Second, another model parameter, $Dc^{2/d}$, gives us a direct estimate of the mean excitation transfer times between different pigment–protein complexes provided that we know the internal composition of the studied PSII fractions. Interestingly, Figure 5a provides independent support for the TTL regime of excitation energy transfer within PSII:[15] for a high detergent concentration the mean excitation hopping time from CP43/CP47 complexes to the RC is about 53 ps (see Figure 5a for the core complexes). However, upon increasing the antenna size, the mean excitation hopping time decreases quickly. This effect demonstrates much better energetic connectivity between the major antenna complexes as compared to the interconnectivity of the core complexes: as the antenna grows, the influence of the intracore migration time becomes less pronounced, leading to the smaller values of the excitation hopping time being averaged over the whole PSII. For the largest PSII supercomplexes, B11, we obtain $\tau_h \approx 25$ ps. When the detergent is removed (0.001% α-DM), the core complexes get into better contact, resulting in a noticeably faster excitation transfer rate. For this low detergent concentration, the mean intercomplex

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excitation hopping times are within the range 20−30 ps, again very close to $\tau_h \approx 25$ ps. This value, in turn, is very close to the excitation migration time scale obtained from the singlet−singlet annihilation measurements performed on LHCII aggregates.[24] Moreover, the same value (25 ps) was obtained in recent structure-based calculations[37] for the intermonomer excitation transfer within the LHCII trimer. Here we would also like to note that (although being indirectly supported by experimental and theoretical works mentioned above) the obtained values for the mean hopping rates in PSII supercomplexes are about 3−5 times smaller than those used for previous coarse-grained modeling.[31−34] As has been mentioned in those studies, some uncertainty in determining the correct τ_h value is present, indicating the sensitivity of the CG model to the way the parameters are defined.

In the case of the BBY particles, we obtain an almost twice as slow mean hopping time, $\tau_h \approx 48$ ps, as for the B11 fractions. Taking into account the quasistacked structural arrangement of antenna complexes in the BBY preparations, this result predicts much slower rates of interlayer excitation migration, raising the total average hopping time. In fact, the overall efficiency of excitation energy transfer between different layers of the thylakoid membrane is still unknown. Therefore, we believe that a direct application of our approach to the time-resolved fluorescence measurements of the whole chloroplast might in principle provide some insight into this problem.

Another important outcome of our simulations is that we manage to fit all the fluorescence kinetics just by assuming infinitely fast excitation trapping by RCs. This implies that the overall process of light harvesting and a subsequent charge separation would be *migration-limited*. Of course, this is not entirely true for the core complexes; indeed, for them our fitted fluorescence kinetics was of slightly worse quality when compared to other fitting results, and this has probably led to some overestimation of the mean hopping time in these core complexes. To deal with these drawbacks, our diffusion model can, in principle, be slightly modified by taking into account a finite rate of photochemical excitation trapping. The details are presented in part III of the Supporting Information. However, this approach would just complicate the analysis of the experimental data and hardly improve the quality of already well-fitted kinetics. Therefore, we would just like to mention that, according to this modified model and in agreement with the existing theoretical treatments,[1] the mean excitation lifetime is determined by the first and the last terms in eq 1, where τ_{mig} is the migration term given by eqs 16 or 19 and $\tau_{trap} = N/\gamma$ is the trapping term depending on the antenna size, N, and the rate of the charge separation, γ. Accordingly, a fast but still finite trapping rate would not change our basic conclusions on the fractional dimensionality of the studied systems and the importance of "*fluctuating bridges*" of excitation energy transfer but just lead to a slightly smaller migration term. Equation 19 then suggests a slight decrease of the mean hopping time τ_h and/or increase of the system's dimensionality d.

While considering the mean excitation lifetime, we can turn back to eq 19 for a moment, now treating N as the number of Chls *a* molecules in the given PSII and τ_h as an average intermolecular excitation hopping time. In the experimental measurements of fluorescence decay kinetics[34] it was observed that the mean excitation lifetime linearly depends on the number of Chl *a* per PSII. However, this dependence does not cross zero when extrapolating toward $N \to 0$ (see Figure 2), as it obviously should do.[1] As already mentioned, such a behavior cannot be understood from the point of view of any existing model of PSII; nevertheless, our proposed diffusion-based fluctuating antenna model can clarify this puzzle. As follows from eq 19, the mentioned experimentally observed linear dependency $\langle\tau(N)\rangle$ is just a projection of the multivariable function $\langle\tau(N,d)\rangle$ onto a single axis N. For any given dimensionality d we have a proper relation $\langle\tau(N\to0,d)\rangle \to 0$, as demonstrated in Figure 6.

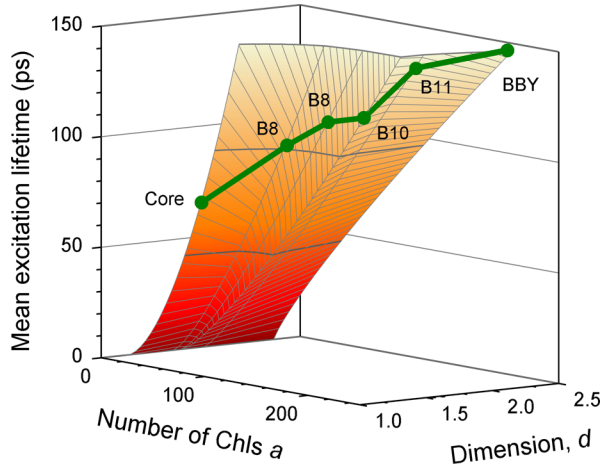

Figure 6. Mean excitation lifetime vs number of Chl *a* molecules and the obtained dimensionality of different fractions of PSII supercomplexes with 0.001% α-DM. Green circles correspond to the exact lifetimes and structural composition of these PSIIs, whereas the d = const gray lines depicted on the three-dimensional surface $\langle\tau(N,d)\rangle$ demonstrate the $\langle\tau(N)\rangle$ behavior for any given d, approaching zero as $N \to 0$.

As a result, our currently presented migration−limited model suggests that the origin of the nonexponential behavior of the fluorescence decay kinetics might be not the reaction center but the fluctuating antenna surrounding it. Accordingly, this outcome allows us to have a fresh view on the results of the recently performed transient absorption measurements of intact PSII core complexes and the isolated intact RCs.[53] The presentation of the kinetics data by lifetime distribution maps (cf. Figures 1 and 2 in ref 53) shows the striking difference in nonexponential features of the RC and core complex at longer times when both systems should exhibit a similar radical pair equilibration process, if any. The lifetime distribution map of the core complex, in contrast to that of the RC, basically shows almost a continuous lifetime distribution over 2 orders of magnitude, which is a characteristic feature of the stretched exponential decay.

6. CONCLUDING REMARKS

In this work we propose a simple *conceptual* model of a fluctuating antenna by describing diffusion of excitation in a continuous medium, intrinsically taking into account varying intercomplex connectivity and different pathways of excitation migration toward the RC. The fluctuating nature and heterogeneity of the light-harvesting systems, caused by the overall membrane dynamics, e.g., needed for the repair of RCs and other self-regulating processes, therefore have a large influence on the exciton dynamics and cannot be underestimated. Despite its simplicity, our approach provides a perfect description of the multiexponential fluorescence decay

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kinetics using just two parameters and without assuming radical pair equilibration in RC. This result implies that the complex multiexponential decay behavior of the fluorescence kinetics might arise from the intrinsic fluctuating properties of the light-harvesting antenna and not from the RC, as is currently broadly accepted. Moreover, it also naturally solves the apparent problem that the excitation mean lifetime does not extrapolate to zero in the case of vanishing antenna size. Additionally, our model is able to provide some valuable information on the structural organization of the photosynthetic antenna, like the stacked structure of BBY complexes with the existing channels for the interlayer excitation energy transfer. We therefore believe that this work, presenting an alternative way to explain known experimental results and to solve some existing discrepancies, will inspire more detailed future simulations and broaden the current understanding of light-harvesting in PSII.

■ ASSOCIATED CONTENT

Supporting Information

Details on the solution of the diffusion equation, an asymptotic expression for the long-time excitation kinetics in PSII, and the influence of the finite trapping rate by RC. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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